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Peculiarities of the dynamics of indices of the age-related transformations of bone metabolism in oral fluid

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The work aimed to establish the patterns of changes of bone tissue metabolism markers in the oral fluid of children and adult patients. There were examined 136 practically healthy individuals aged 6 to 89 years and the following groups have been formed: primary school age patients (group 1, 6-10 years, n = 16), adolescents (group 2, 11-17 years, n = 17), juvenile age patients (group 3, 18-24 years, n = 25), young adults (group 4, 25-44 years old, n = 21), middle-aged adults (group 5, 45-59 years old, n = 22), elderly adults (group 6, 60-74 years old, n = 19) and senile patients (group 7, 75-89 years old, n = 16). Oral fluid was obtained using a non-stimulatory method, and biochemical indices were determined in accordance with standardised laboratory techniques, following the ethical principles of conducting scientific medical research involving human participants. It has been established that periods of particular risk are primary school and middle age. In primary school-age children and adolescents, the physiological processes of bone growth and remodelling prevail. Between the ages of 6 and 10, these processes are accompanied by a 33% increase in ionised calcium content and a 41% increase in acid phosphatase activity, reflecting controlled bone resorption during teething. In middle-aged patients, a significant imbalance of bone metabolism transformation indexes (activation of acid and alkaline phosphatases by 31 and 32%, respectively) is a trigger for rapid progression of inflammatory and destructive periodontal processes in the subsequent age periods, and the lack of proper dispensary observation by a dentist can lead to tooth loss. It is important that at the age of 45-59, calcium homeostasis is significantly influenced by the pH of oral fluid. A strong inverse correlation has been established between the content of ionised calcium and the pH of oral fluid in middle-aged patients (r = -0.79), while in other age groups this correlation was moderate (r = -0.49 – -0.65). The obtained data justify the need to monitor the indexes of calcium and phosphate metabolism of oral fluid at different age periods and the advantages of an age-oriented approach to the prevention and personalised planning of dental treatment.

Key words: oral fluid; age-related changes; bone remodeling; calcium homeostasis; markers of bone metabolism; alkaline phosphatase; acid phosphatase; mineral metabolism; periodontitis.

INTRODUCTION

The maintenance of mineral homeostasis within the oral cavity is a key factor in sustaining the structural-functional integrity of enamel, the condition of hard and soft tissues, as well as the resistance of teeth to the caries and periodontal diseases. The balance of calcium (both total and ionised), the activity of enzymes of the calcium-phosphorus exchange, which determine the intensity of demineralisation and remineralisation processes, are of a particular importance in this process [1, 2]. The age-related changes observed in these indices reflect physiological processes and possible compensatory reactions of the oral cavity organs.

Oral fluid, regarded as a universal diagnostic medium, is sensitive to the age-related metabolic changes in the body. During the ageing process, the concentration of minerals changes, as well as the enzymatic activity and protein composition of saliva, affecting its buffering properties, protective potential and oral microbiome [3]. Calcium homeostasis is one of the key regulatory mechanisms that ensure the structural integrity

and functional stability of oral tissues. Calcium plays a crucial role in the mineralisation of hard tooth components, thereby maintaining the physiological state of the alveolar process/part, as well as in regulating the secretory activity of the salivary glands and the oral fluid protective properties. Ionised calcium, as the active form of the mineral, plays a pivotal role in the formation of pellicle, enamel mineralisation and biofilm dynamics, while total calcium reflects the total mineral reserve of oral fluid [2, 4]. A calcium imbalance is associated with the development of caries, non-carious lesions of hard tooth tissues, periodontal diseases, and a decrease in the reparative potential of the oral mucosa [5, 6]. The activity of alkaline phosphatase (ALP) and acid phosphatase (ACP) enzymes regulates phosphate metabolism and hard tissue remodelling; increases in the activity of these enzymes in specific age groups may signal the activation of bone metabolism processes and the restructuring of periodontal structures. With age, there are some changes in the concentration of oral fluid proteins, affecting calcium transport, the stability of calcium-phosphate complexes, and the functioning of local defence mechanisms [1, 7].

Despite the significant number of studies devoted to the biochemical characteristics of oral fluid/saliva, the issue of age-related peculiarities of calcium homeostasis in the oral cavity has not been studied systematically enough. Concurrently, modern dental science and practice are more likely to encounter the pathological processes that have already been diagnosed; while there is no basis for early monitoring of indices of age-related changes in the oral fluid bone metabolism, which would allow the detection of pathological processes in the dentoalveolar system even before the appearance of clinical and radiological signs. The relevance of the issue is reinforced by the necessity to develop the age-oriented preventive measures, to understand the mechanisms of increased cariogenicity or susceptibility to periodontal diseases, as well as osteoporosis of the alveolar process/part at different ages.

The objective of this study is to identify patterns of changes in markers of bone tissue metabolism in the oral fluid of children and adult patients.

METHODS

The study involved 136 patients aged 6 to 89 years without general somatic and dental pathologies, who considered themselves to be practically healthy. According to the classification system of age categories (WHO, 2015), the following groups were formed: primary school age patients (group 1, aged 6-10 years, n = 16), adolescents (group 2, aged 11-17 years old, n = 17), juvenile age patients (group 3, aged 18-24 years old, n = 25), young adults (group 4, aged 25-44 years old, n = 21), middle-aged adults (group 5, aged 45-59 years old, n = 22), elderly patients (group 6, aged 60-74 years old, n = 19) and senile patients (group 7, aged 75-89 years old, n = 16). An objective dental examination was performed using a standard set of instruments at the clinic of the Dental Centre of Ivano-Frankivsk National Medical University (IFNMU). The examinations were conducted with the informed consent of patients for clinical research, which was approved by the IFNMU Ethics Committee (Protocol No. 125/22, dated March 25, 2022). The study was performed in compliance with the World Medical Association's Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects (1964-2013) and Order № 690 of the Ministry of Health of Ukraine dated September 23, 2009 (as amended by Order of the Ministry of Health of Ukraine № 523 dated July 12, 2012). Oral fluid was obtained by spitting into sterile test tubes in the morning, on an empty stomach, without stimulation of salivation, prior cleaning or rinsing of the oral cavity. The peculiarities of bone remodelling were characterised by the following factors: the concentration of total and ionised calcium [8], the activity of ALP and ACP [9], the content of total protein in oral

fluid [10], and the acid-base balance index [11]. The biochemical studies were performed at the Centre for Bioelementology, which is part of the IFNMU.

The statistical processing of the results was performed using Microsoft Office 365 ProPlus Excel with the application of variational statistics methods using Student's t-test. The normality of the distribution of the obtained data was checked using Shapiro-Wilk's W test. As all indices complied with the law of normal distribution, an interval (M ± m) was used for the description of the central tendency (typical values). The difference between the studied indices was considered to be of significance at a value of $P < 0.05$. The strength and direction of the relationship between the indices was assessed using the Pearson parametric correlation coefficient (r) by means of correlation analysis method.

RESULTS AND DISCUSSION

In the oral fluid of the examined patients in group 1 (primary school age), calcium metabolism and bone remodelling markers reflected age-related peculiarities of intensive growth and bone tissue formation. An increase in ionised calcium levels and ACP activity relative to the reference values was a characteristic feature of children in this age group (Fig. 1A, B). The increase in these indices reflects the intensive remodelling of the bone structures of the alveolar process/part associated with the physiological eruption of permanent teeth during the period of mixed dentition. The data obtained are consistent with the physiologically determined processes of growth of the maxillofacial apparatus and active bone remodelling in childhood, which includes both resorption and bone formation aimed at ensuring the harmonious development of the dentofacial system [12, 13].

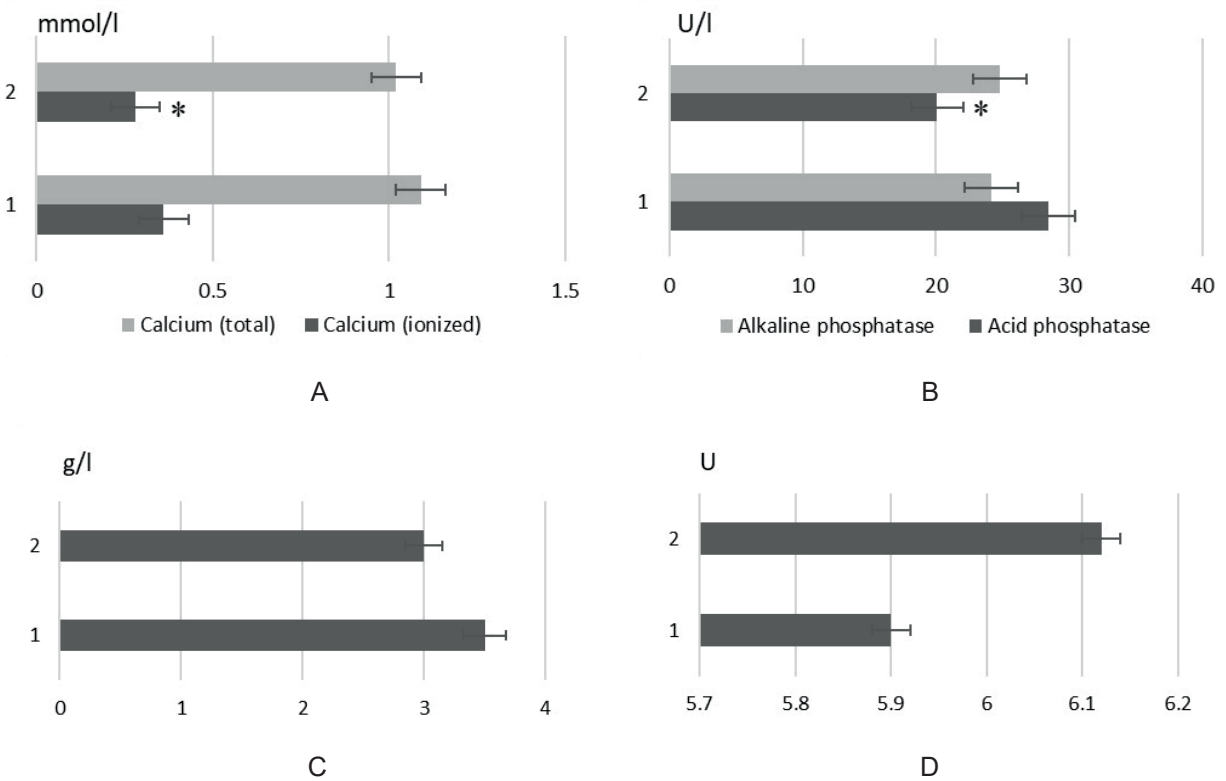

Fig. 1. Age-related changes in the content of total and ionized calcium (A), the activity of acid and alkaline phosphatases (B), total protein content (C), and pH (D) in oral fluid of children (M ± m): 1 – primary school age (6-10 years), 2 – adolescents (11–17 years); *$P < 0.05$ – compared to group 1

In patients of the group 2 (adolescent age), the studied indices remained relatively stable, indicating a gradual transition from predominantly anabolic processes in childhood to more balanced bone remodelling process. The absence of significant fluctuations in calcium concentration and phosphatase activity reflects the adaptation of regulatory mechanisms to hormonal and metabolic changes characteristic of puberty. Such peculiarities can be considered as a physiological restructuring of the mineral metabolism system preceding adolescence. At the same time, in the examined primary school children, the content of ionised calcium and the activity of ACP in oral fluid exceeded the values in adolescents at 32.67 and 41.25%, respectively ($P_{1-2} < 0.05$), which may be due to the age-related peculiarities of the period of mixed dentition (see Fig. 1A, B). Such changes are a reflection of the intensive processes of mineralisation and physiological remodelling of the alveolar process/part. It is important that the level of ionised calcium and the activity of bone metabolism enzymes (particularly ACP) play a significant role in the formation of orthognathic occlusion, harmonious development of the maxillofacial complex and articulatory apparatus [14]. The adequacy of these indices in early school age creates favourable conditions for the correct eruption of permanent teeth, the formation of occlusal relationships, and the functional maturity of the speech apparatus in further ontogenesis. The level of total protein in the oral fluid of the examined of the first and second groups did not reliably change and remained within the normal range (see Fig. 1C). The same tendency was found when comparing the indices of the groups 3 and 4 (juvenile and young age, respectively) (see Figs. 1; 2). Therefore, it can be claimed that the transition from adolescence to adulthood is not accompanied by significant changes in calcium concentrations or enzyme activity in oral fluid that regulate mineral metabolism. Calcium metabolism in this age group is relatively stable.

In patients of group 5 (middle age), the concentration of total and ionised calcium did not differ significantly from the values observed in groups 3 and 4. Concurrently, ALP activity increased at 31.55%, ACP at 31.31% ($P_{3-5} < 0.05$), and the total protein content in oral fluid demonstrated a 52.54% increase ($P_{3-5} < 0.05$) in comparison to the values of similar indices observed in juvenile age patients. Such peculiarities are indicative of a compensatory response to age-related changes in the oral cavity, when enzyme systems are activated earlier than a noticeable shift in the concentration of essential minerals occurs. The body detects the onset of destructive and reparative processes in tissues, as confirmed by an increase in ALP and ACP, but successfully compensates for calcium loss, preventing its probable changes in the oral fluid. An increase in total protein content is the primary sign of the onset of age-related changes in the structure and function of the salivary glands, leading to hyposalivation and increased inflammatory processes in the periodontal tissues [15-17].

In the oral fluid of the examined in the group 6 (elderly patients), the total calcium content increased 2.11-fold ($P_{3-6} < 0.01$) in comparison with the values observed in the group 3, while the changes in ionised calcium proved to be non-significant. This dynamic was determined against the background of an increase in ALP and ACP activity at 61.94 and 68.60%, respectively ($P_{3-6} < 0.01$) compared to the values in the examined juvenile age patients. At the same time, the levels of total and ionised calcium in the oral fluid of patients in group 6 exceeded the values in group 4 at 60.00 and 42.31%, respectively ($P_{4-6} < 0.01$) against the background of ALP and ACP activation at 65.22 and 80.82%, respectively ($P_{4-6} < 0.01$). The total protein content in the oral fluid of elderly patients exceeded the values in adolescents and young people 2.10-fold ($P_{3-6} < 0.01$) and at 57.76% ($P_{4-6} < 0.01$), respectively; but these values did not undergo significant changes when compared to the values in middle-aged people. It can be assumed that there is a gradual depletion of compensatory mechanisms

and a predominance of destructive processes in tissues, resulting in a significant release of calcium into oral fluid and the progression of tissue demineralisation and resorption processes. Osteoporosis is a condition that typically progresses intensively in old age, particularly after the age of 60. Bone tissue (especially the alveolar process/part) undergoes accelerated resorption, releasing significant quantities of minerals into the blood and subsequently into the oral fluid. Against the background of a decrease in the protective function of saliva and the accumulation of dental plaque (due to inadequate hygiene), the demineralisation of dental tissues accelerates, which further increases the calcium content in oral fluid [18].

Ionised calcium is a biologically active form of crucial importance for physiological

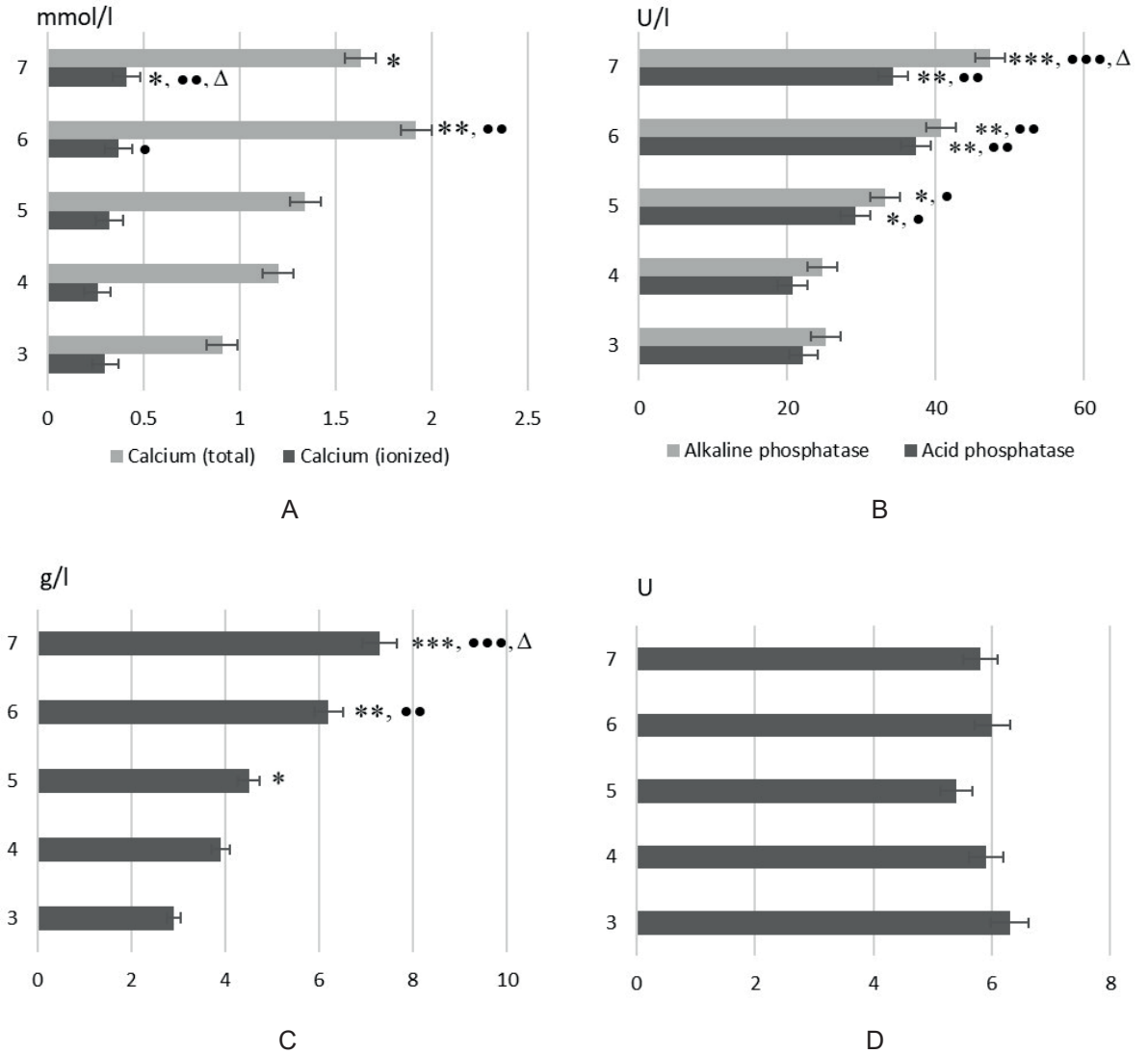

Fig. 2. Age-related changes in the content of total and ionized calcium (A), the activity of acid and alkaline phosphatases (B), total protein content (C), and pH (D) in oral fluid of adult patients (M ± m): 3 – juvenile age patients (18–24 years), 4 – young adults (25-44 years), 5 – middle-aged adults (45-59 years), 6 – elderly adults (60-74 years), 7 – senile (75–89 years); reliable differences; *P < 0.05, **P < 0.01, ***P < 0.001 – compared to group 3; •P < 0.05, ••P < 0.01, •••P < 0.001 – compared to group 4, Δ P < 0.05 – compared to group 5

processes in cells. Under physiological conditions, its reference values are maintained within narrow limits. A significant increase in the index values indicates the strain on compensatory mechanisms. An excess of calcium intake results in the body's inability to bind and excrete it, which leads to an increase in the level of the active, ionised form [19]. The activation of oral fluid phosphatases in patients in group 6 confirms that this is the age at which destructive processes reach their peak. Excessive activation of ALP may be a reaction to the increased bone remodelling (an attempt at repair against the background of osteoporosis) and progressive periodontitis, while ACP reflects high osteoclast activity and the progression of destructive processes in periodontal tissues. The development of the above-mentioned age-related changes causes chronic inflammation, gland atrophy and leads to an increase in protein content in oral fluid [20-22].

The absence of reliable differences between the studied indices in groups 5 and 6 indicates that fundamental pathogenetic processes (the onset of bone resorption, enzyme activation) begin to progress from middle to old age. We tend to believe that the onset of the highest level of bone resorption in old age is due to the processes that began at the age of 45-59.

In the oral fluid of patients in group 7 (senile), the content of total and ionised calcium exceeded the values in group 3 at 79.12 and 36.67% ($P_{3-7} < 0.05$), the activity of ALP and ACP increased at 87.70 ($P_{3-7} < 0.001$) and at 54.50% $P_{3-7} < 0.01$) (Fig. 2A, B). The same tendency was observed in group 4 (the content of ionised calcium increased at 57.69%, $P_{4-7} < 0.01$, ALP and ACP activity increased at 91.50%, $P_{4-7} < 0.001$ and at 65.70%, $P_{4-7} < 0.01$, respectively). However, these differences in total calcium were not significant. In comparison with the values of the group 5, only ALP activation was determined (at 42.68%, $P_{5-7} < 0.05$), while the differences between the values of calcium metabolism indices in the examined from groups 6 and 7 were not found to be significant. At the same time, in senile patients, the total protein content in oral fluid exceeded the values in juvenile age patients 2.47-fold ($P_{3-7} < 0.001$) young adults – at 85.49% ($P_{4-7} < 0.001$) and middle-aged adults – at 62.00% ($P_{5-7} < 0.05$). Such dynamics may characterise the culmination of age-related pathological processes in senile age due to the depletion of compensatory mechanisms in the body. The maintenance of the concentration of ionised calcium in the oral fluid within the narrow limits of the examined patients from groups 3-6, can be achieved through the buffer systems and its binding to proteins. Under physiological conditions, this property is depleted exclusively among individuals over the age of 75. An increase in the total protein content in oral fluid and atrophy of the salivary glands lead to hyposalivation and changes in the composition of saliva. These processes impair the ability of proteins to effectively bind calcium ions, resulting in a progressive increase in its active form. The maximum activation of oral fluid phosphatases is a marker of the highest activity of remodelling processes, accompanied by intense tissue destruction. In the senile age, diseases such as chronic periodontitis and osteoporosis are in their late stages, requiring a constant but often ineffective reparative response from the body (increase in ALP) in response to large-scale bone resorption and inflammation (activation of ACP).

Attracts attention that the acid-base balance of oral fluid shift towards acidity in primary school, middle-aged, elderly and senile age, which contributes to the demineralisation of hard tooth tissues (see Figs. 1; 2D). The study revealed a robust inverse correlation ($r = -0.79$) between ionised calcium content and oral fluid pH in the middle-aged patients. It can be argued that a decrease in the acidity of oral fluid significantly affects the increase in calcium content in patients of this age group, which may reflect the age-related peculiarities inherent in the regulation of the mineral composition and buffer properties of saliva. In patients of other age groups, the correlation between

calcium content and oral fluid pH was moderate (inverse, moderate strength, r = -0.49 to -0.65). Discrepancies in the strength of the relationship between mineral metabolism indices and acid-base balance in different groups may be due to both physiological and metabolic changes that occur in the body at different ages, including hormonal profile, eating habits, and general somatic condition.

The results of the study characterise the potential of oral fluid mineral indices (calcium content and pH value) as biomarkers of the risk of developing dental pathologies such as caries, periodontitis and hyposalivation, as well as the indices of age-related systemic metabolic disorders. It is vital to take into account the age-related peculiarities of the mineral composition of oral fluid in order to achieve a more accurate risk assessment, which,in turn, opens up prospects for early diagnosis and individualisation of preventive and therapeutic measures. In general, changes in the mineral composition and acid-base balance of the oral fluid in different age groups can serve as informative markers of both local dental processes and general somatic disorders, which justifies their use in clinical-diagnostic practice.

Consequently, a distinct age-related dynamic of calcium homeostasis indices and bone remodelling markers in oral fluid has been determined. In children of primary school and adolescent age (6-17 years old), physiological processes of bone tissue growth and remodelling prevail, which at the age of 6-11 years are accompanied by an increase in the content of ionised calcium and ACP activity, reflecting controlled bone resorption during tooth eruption. These changes occur under conditions of preserved mineral balance and show no signs of pathological destructive processes. In juvenile age and young adulthood, calcium metabolism indices attain adult reference values, reflecting state of equilibrium between the processes of bone formation and resorption. The age group deemed to be at risk consists of middle-aged patients, as they experience increased activity of bone remodelling enzymes against a background of preserved calcium homeostasis, reflecting the activation of compensatory mechanisms in response to initial changes in oral tissues. In elderly and senile age patients, there is a progressive disorder of mineral metabolism with excessive activation of ALP and ACP, an increase in calcium content, reflecting the depletion of the body's compensatory capabilities and the predominance of destructive and inflammatory processes in the periodontal tissues. The dental status of patients in these groups is determined by the degree of preservation of the physiological processes of bone metabolism in previous age periods.

CONCLUSIONS

Age-related changes in oral bone metabolism have their own peculiarities, which are determined by hormonal and metabolic profiles. Periods of particular risk are: primary school age – changes at this time can cause abnormalities in the dentition and speech apparatus (increased levels of ionised calcium and ACP activity), middle age – the most rapid imbalance in bone metabolism transformation indices triggers the rapid progression of inflammatory-destructive processes in the periodontium. Furthermore, the lack of proper dispensary observation by a dentist can lead to tooth loss. The pH of oral fluid has a significant impact on bone remodelling processes, particularly in individuals aged 45-59. The data obtained substantiate the importance of monitoring the indices of calcium and phosphate metabolism in oral fluid at different age periods as potential age-oriented biomarkers of caries, periodontitis and mineral metabolism disorders within bone tissue.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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ОСОБЛИВОСТІ ДИНАМІКИ ІНДИКАТОРІВ ВІКОВИХ ТРАНСФОРМАЦІЙ КІСТКОВОГО МЕТАБОЛІЗМУ РОТОВОЇ РІДИНИ

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Мета нашої роботи – встановити закономірності змін маркерів метаболізму кісткової тканини ротової рідини дітей і дорослих пацієнтів. Обстежено 136 практично здорових осіб віком від 6 до 89 років і сформовано такі групи: молодший шкільний вік (1-ша, 6 – 10 років, n = 16), підлітковий (2-га, 11 – 17 років, n = 17), юнацький (3-тя, 18 – 24 роки, n = 25), молодий (4-та, 25 – 44 роки, n = 21), середній (5-та, 45 – 59 років, n = 22), похилий (6-та, 60 – 74 роки, n = 19) і старечий (7-ма, 75 – 89 років, n = 16) вік. Ротову рідину отримували безстимуляційним методом, біохімічні показники визначали відповідно до стандартизованих лабораторних методик. Встановлено, що періодами особливого ризику є молодший шкільний і середній вік. У дітей молодшого шкільного та підліткового віку переважали фізіологічні процеси росту та перебудови кісткової тканини, які у віці 6–10 років супроводжувалися підвищенням вмісту іонізованого кальцію (на 33 %) та активності кислої фосфатазаи (на 41 %), що відображає контрольовану резорбцію кістки під час прорізування зубів. У пацієнтів середнього віку суттєвий дисбаланс показників трансформацій кісткового метаболізму (підвищення активності кислої та лужної фосфатаз на 31 та 32 % відповідно) є тригером швидкого прогресування запально-деструктивних процесів пародонта у наступних вікових періодах, а відсутність належного диспансерного спостереження у лікаря-стоматолога може призводити до втрати зубів. Важливо, що у віці 45–59 років на кальцієвий гомеостаз суттєво впливає pH ротової рідини. Установлено сильний, зворотний кореляційний взаємозв'язок вмісту іонізованого кальцію та значення pH ротової рідини у пацієнтів середнього віку (r = -0,79), тоді як в інших вікових групах такий зв'язок був середньої сили (r = -0,49 – -0,65). Отримані результати обгрунтовують необхідність моніторингу показників кальцієвого і фосфатного обміну ротової рідини у різні вікові періоди та переваги підходу, орієнтованого на вік до профілактики і персоналізованого планування стоматологічного лікування.

Ключові слова: ротова рідина; вікові зміни; кісткове ремоделювання; кальцієвий гомеостаз; маркери кісткового метаболізму; лужна фосфатаза; кисла фосфатаза; мінеральний метаболізм; пародонтит.

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